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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **Haugland et al**

Docket No.: **2085**

Serial No.: **10/005,050** ✓

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For: **Labeling of Immobilized Proteins Using Dipyrrometheneboron Difluoride Dyes**

Information Disclosure Statement

Assistant Commissioner for Patents
Washington, D.C. 20231

<p style="text-align: center;">CERTIFICATE OF MAILING</p> <p>I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HERewith ARE BEING DEPOSITED WITH THE UNITED STATES POSTAL OFFICE ON <u>2/28/02</u> AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO : ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON D.C. 20231</p> <p><i>Allegre J. Heffenstein</i> Allegre J. Heffenstein, REG. NO. 34,179</p>

Dear Sir:

In accordance with their duty of disclosure under 37 CFR §§ 1.97 and 1.56, Applicants hereby disclose the following references.

- U.S. Patent No. 5,270,163 Gold et al (1993)
- U.S. Patent No. 4,774,339 Haugland et al (1988)
- U.S. Patent No. 5,187,288 Kang et al (1993)
- U.S. Patent No. 5,248,782 Haugland et al (1993)
- U.S. Patent No. 5,274,113 Kang et al (1993)
- U.S. Patent No. 5,451,663 Kang et al (1995)
- U.S. Patent No. 5,719,031 Haugland et al (1998)
- U.S. Patent No. 4,931,223 Bronstein et al (1990)
- U.S. Patent No. 4,962,192 Schaap (1990)
- U.S. Patent No. 5,338,854 Kang et al (1994)
- U.S. Patent No. 5,433,896 Kang et al (1995)
- U.S. Patent No 6,162,931 Gee et al. (2000)
- U.S. Patent No. 5,443,986 Haugland et al (1995)
- U.S. Patent No. 5,196,306 Bobrow et al (1993)
- U.S. Patent No. 5,583,001 Bobrow et al (1996)
- U.S. Patent No. 5,731,158 Bobrow et al (1998)
- U.S. Patent No. 4,810,636 Corey (1989)
- U.S. Patent No. 5,316,906 Haugland et al. (1994)
- U.S. Patent No. 6,130,101 Mao et al. (2000)

Ann. Rev. Biochem. 64, 763 (1995)

Chem. Rev. 97, 349 (1997)

Anal. Biochem. 164, 303 (1987)

Meth. Mol. Biol. Ch.35, 313 (1999)

Anal. Biochem. 276, 129 (1999)

Anal. Biochem. 202, 100 (1992)

Electrophoresis 21, 2196 (2000)

BioTechniques 30, 266, (2001)

Electrophoresis 21, 1123 (2000)

BioTechniques 28, 944, (2000)

Electrophoresis 19, 2407 (1998)

Electrophoresis 22, 881 (2001)

J. Biochem. Biophys. Methods 46, 31 (2000)

R. Haugland, MOLECULAR PROBES HANDBOOK OF FLUORESCENT PROBES AND RESEARCH CHEMICALS, 6th Edition., (1996) and its subsequent 7th Edition, and 8th Edition updates on CD-ROM in November 1999 and May 2001.

R. Haugland, MOLECULAR PROBES HANDBOOK OF FLUORESCENT PROBES AND RESEARCH CHEMICALS, 6th Edition at p. 14 (1996)

Anal. Biochem. 251, 144 (1997)

U.S. Ser. No. 09/970,215 to Haugland, et al. (2001)

U.S. Ser. No. 09/969,853 to Leung, et al. (2001)

The references listed above are cited in the body of the subject application, along with an indication of their relevance to the subject invention. For the convenience of the Examiner, the references are listed on modified PTO Form 1449 (attached) and copies thereof are enclosed.

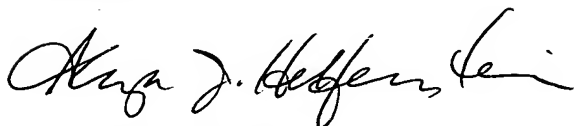
Of the references listed above, the '339, '288, '782, '113, '663, '854, '896 patents specifically describe a variety of dipyrrometheneboron difluoride dyes and derivatives, including dyes used for the practice of the claimed invention. The MOLECULAR PROBES HANDBOOK and CD-ROM references

describe dipyrrometheneboron difluoride dyes and derivatives that are commercialized by Molecular Probes, Inc. (assignee of the subject application) under the trademark BODIPY for a wide variety of biological applications. None of the parents or literature references, however, describe or suggest that when dipyrrometheneboron difluoride dyes are used to covalently label proteins that are attached to a solid support, significant fluorescent quenching is not observed, as was unexpectedly found for the methods of the present invention. In contrast to the subject invention, the Jones reference and '031 patent describe methods for labeling proteins with dipyrrometheneboron difluoride dyes at a concentration sufficient to generate significant fluorescence quenching, such that subsequent enzymatic degradation results in enhanced fluorescence.

The Szewczyk and Berggren references describe general methods for detecting proteins that are immobilized on membranes after electroblotting from polyacrylamide gels, using fluorescent and non-fluorescent stains, as well as some of the associated problems. Such problems include irreversible binding to the membrane (Szewczyk at 304, RESULTS AND DISCUSSION); and the impact of environmental factors (Berggren at 142, first paragraph). The references neither describe nor suggest that such problems can be resolved by the use of dipyrrometheneboron difluoride dyes according to the methods of the invention.

In view of the known art, taken as a whole, Applicants believe that their invention is novel and unobvious. In the event that there are any questions relating to this paper, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that the prosecution of the application may be expedited.

Respectfully submitted,



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